L8: Entry 5 of 16 File: PGPB Sep 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020132320
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020132320 A1

TITLE: Glycoconjugate synthesis using a pathway-engineered organism

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Wang, Peng George | Troy | MI | US | |
| Chen, Xi | Norristown | PA | US | |
| Liu, Ziye | Detroit | MI | US | |
| Zhang, Wei | Detroit | MI | US | |

US-CL-CURRENT: 435/193; 435/101, 435/200, 435/320.1, 435/325

CLAIMS:

1. A vector comprising: (a). two or more genes encoding sugar-nucleotide regenerating enzymes selected from the group consisting of GalK, GalT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, PgM, NagE, Agm1, glmU, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b). one or more genes encoding glycosyltransferase(s), wherein said genes are operably linked to a promoter.

2. The vector of claim 1 comprising genes encoding three or more enzymes for regenerating a sugar-nucleotide.

3. The vector of claim 1 comprising genes encoding two or more glycosyltransferases.

4. The vector of claim 1 comprising genes encoding three or more glycosyltransferases.

5. The vector of claim 1 comprising genes encoding GalK, GalT, and GalU.

6. The vector of claim 5 further comprising a gene encoding Ndk.

7. The vector of claim 5 further comprising a gene encoding Ppk.

8. The vector of claim 5 further comprising a gene encoding PykF.

9. The vector of claim 5 further comprising genes encoding PoxB, Ndk, and Ppa.

10. The vector of claim 1 comprising a gene encoding SusA.

L8: Entry 10 of 16 File: USPT Nov 23, 2004

US-PAT-NO: 6821756
DOCUMENT-IDENTIFIER: US 6821756 B2

TITLE: Processes for reproducing sugar nucleotides and complex carbohydrates

DATE-ISSUED: November 23, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Koizumi; Satoshi | Tokyo | | | JP |
| Sasaki; Katsutoshi | Tokyo | | | JP |
| Endo; Tetsuo | Tokyo | | | JP |
| Tabata; Kazuhiko | Tokyo | | | JP |
| Ozaki; Akio | Tokyo | | | JP |

US-CL-CURRENT: 435/89; 435/183, 435/190, 435/193, 435/194, 435/252.3, 435/252.32, 435/253.6, 435/320.1, 435/325, 435/4, 435/41, 435/6, 435/69.1, 530/350, 536/23.2, 536/23.4, 536/23.7

CLAIMS:

What is claimed is:

1. A process for producing guanosine diphospho-sugar ("GDP-sugar") or uridine diphospho-sugar ("UDP-sugar"), which comprises: selecting, as enzyme sources, a) a culture of a microorganism capable of producing guanosine-5'-triphosphate ("GTP") or uridine-5'-triphosphate ("UTP") from a nucleotide precursor, or a treated product of the culture selected from the group consisting of a concentrated product of the culture, a dried product of the culture, a culture supernatant obtained by centrifuging the culture, a concentrated product of the culture supernatant, an enzyme preparation obtained from the culture supernatant, cells obtained by centrifuging the culture, a dried product of the cells, a freeze-dried product of the cells, a surfactant-treated product of the cells, an ultrasonic-treated product of the cells, a mechanically disrupted product of the cells, a solvent-treated product of the cells, an enzyme-treated product of the cells, a protein fraction of the cells, an immobilized product of the cells and an enzyme preparation obtained by extraction from the cells, and b) a culture or cultures of at least one strain of microorganism having genes responsible for production of GDP-sugar or UDP-sugar from a sugar selected from the group consisting of glucose, fructose, galactose, glucosamine, N-acetylglucosamine, N-acetylgalactosamine, mannose and fucose and GTP or UTP, or a treated product of the culture selected from the group consisting of a concentrated product of the culture, a dried product of the culture, a culture supernatant obtained by centrifuging the culture, cells obtained by centrifuging the culture, a dried product of the cells, a freeze-dried product of the cells, a surfactant-treated product of the cells, a solvent-treated product of the cells, and an immobilized product of the cells wherein the treated product of the culture continues to have the same

enzymatic activity as said culture capable of producing UDP-sugar or GDP-sugar from the sugar and UTP or GTP; allowing the enzyme sources, the nucleotide precursor and the sugar to be present in an aqueous medium to form and accumulate GDP-sugar or UDP-sugar in the aqueous medium; and recovering GDP-sugar or UDP-sugar from the aqueous medium.

2. The process according to claim 1, wherein the nucleotide precursor is orotic acid, uracil, orotidine, uridine, cytosine, cytidine, adenine, adenosine, guanine, guanosine, hypoxanthine, inosine, xanthine, xanthosine, inosine-5'-monophosphate, xanthosine-5'-monophosphate, guanosine-5'-monophosphate, uridine-5'-monophosphate or cytidine-5'-monophosphate.

3. The process according to claim 1, wherein the microorganism capable of producing GTP or UTP from a nucleotide precursor is a microorganism selected from microorganisms belonging to the genus Corynebacterium.

4. The process according to claim 3, wherein the microorganism belonging to the genus Corynebacterium belongs to Corynebacterium ammoniagenes.

5. The process according to claim 1, wherein the at least one strain of microorganism having genes responsible for production of a sugar nucleotide comprises a recombinant microorganism having at least one gene responsible for production of a sugar nucleotide, said gene being derived from a different microorganism, or being derived from said strain of microorganism but being harbored in a plasmid.

6. The process according to claim 5, wherein the recombinant microorganism is selected from microorganisms belonging to the genus Escherichia and the genus Corynebacterium.

7. The process according to claim 6, wherein the recombinant microorganism is Escherichia coli.

8. The process according to claim 6, wherein the recombinant microorganism is Corynebacterium ammoniagenes.

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**Search Results -** Record(s) 1 through 10 of 16 returned.

☐ 1. Document ID: US 20030186414 A1

**Using default format because multiple data bases are involved.**

L8: Entry 1 of 16 File: PGPB Oct 2, 2003

PGPUB-DOCUMENT-NUMBER: 20030186414
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030186414 A1

TITLE: Nucleic acid that encodes a fusion protein

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Gilbert, Michel | Hull | | CA | |
| Young, N. Martin | Gloucester | | CA | |
| Wakarchuk, Warren W. | Gloucester | | CA | |

US-CL-CURRENT: 435/193; 435/320.1, 435/325, 435/6, 435/69.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 2. Document ID: US 20030180928 A1

L8: Entry 2 of 16 File: PGPB Sep 25, 2003

PGPUB-DOCUMENT-NUMBER: 20030180928
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030180928 A1

TITLE: Fusion protein comprising a UDP-Galnac 4' epimerase and a galnac transferase

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Gilbert, Michel | Hull | | CA | |
| Young, N. Martin | Gloucester | | CA | |
| Wakarchuk, Warren W. | Gloucester | | CA | |

US-CL-CURRENT: 435/193; 435/320.1, 435/325, 435/6, 435/69.7, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 3. Document ID: US 20030082511 A1

L8: Entry 3 of 16 File: PGPB May 1, 2003

PGPUB-DOCUMENT-NUMBER: 20030082511
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030082511 A1

TITLE: Identification of modulatory molecules using inducible promoters

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Brown, Steven J. | San Diego | CA | US | |
| Dunnington, Damien J. | San Diego | CA | US | |
| Clark, Imran | San Diego | CA | US | |

US-CL-CURRENT: 435/4; 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 4. Document ID: US 20020150968 A1

L8: Entry 4 of 16 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020150968
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020150968 A1

TITLE: Glycoconjugate and sugar nucleotide synthesis using solid supports

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Wang, Peng G. | Troy | MI | US | |
| Chen, Xi | Norristown | PA | US | |

US-CL-CURRENT: 435/53; 435/175, 435/68.1, 435/96

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 5. Document ID: US 20020132320 A1

L8: Entry 5 of 16 File: PGPB Sep 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020132320
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020132320 A1

TITLE: Glycoconjugate synthesis using a pathway-engineered organism

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Wang, Peng George | Troy | MI | US | |
| Chen, Xi | Norristown | PA | US | |
| Liu, Ziye | Detroit | MI | US | |
| Zhang, Wei | Detroit | MI | US | |

US-CL-CURRENT: 435/193; 435/101, 435/200, 435/320.1, 435/325

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 6. Document ID: US 20020064836 A1

L8: Entry 6 of 16 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064836
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064836 A1

TITLE: PROCESSES FOR PRODUCING SUGAR NUCLEOTIDES AND COMPLEX CARBOHYDRATES

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| KOIZUMI, SATOSHI | TOKYO | | JP | |
| SASAKI, KATSUTOSHI | TOKYO | | JP | |
| ENDO, TETSUO | TOKYO | | JP | |
| TABATA, KAZUHIKO | TOKYO | | JP | |
| OZAKI, AKIO | TOKYO | | JP | |

US-CL-CURRENT: 435/89; 435/170, 435/255.1, 435/41, 435/88, 435/92

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 7. Document ID: US 20020034805 A1

L8: Entry 7 of 16 File: PGPB Mar 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020034805
PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020034805 A1

TITLE: FUSION PROTEINS FOR USE IN ENZYMATIC SYNTHYESIS OF OLIGOSACCHARIDES

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| GILBERT, MICHEL | HULL | | CA | |
| YOUNG, N. MARTIN | GLOUCESTER | | CA | |
| WAKARCHUK, WARREN W. | GLOUCESTER | | CA | |

US-CL-CURRENT: 435/193; 435/183, 435/200, 435/320.1, 435/325, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 8. Document ID: US 20020025560 A1

L8: Entry 8 of 16 File: PGPB Feb 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020025560
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020025560 A1

TITLE: Processes for producing sugar nucleotides and complex carbohydrates

PUBLICATION-DATE: February 28, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Koizumi, Satoshi | Tokyo | | JP | |
| Sasaki, Katsutoshi | Tokyo | | JP | |
| Endo, Tetsuo | Tokyo | | JP | |
| Tabata, Kazuhiko | Tokyo | | JP | |
| Ozaki, Akio | Tokyo | | JP | |

US-CL-CURRENT: 435/89; 435/91.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 9. Document ID: US 20020001831 A1

L8: Entry 9 of 16 File: PGPB Jan 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020001831
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020001831 A1

TITLE: Low cost manufacture of oligosaccharides

PUBLICATION-DATE: January 3, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Defrees, Shawn | North Wales | PA | US | |
| Johnson, Karl | Willow Grove | PA | US | |

US-CL-CURRENT: 435/101; 435/84, 536/53

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 10. Document ID: US 6821756 B2

L8: Entry 10 of 16 File: USPT Nov 23, 2004

US-PAT-NO: 6821756
DOCUMENT-IDENTIFIER: US 6821756 B2

TITLE: Processes for reproducing sugar nucleotides and complex carbohydrates

DATE-ISSUED: November 23, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Koizumi; Satoshi | Tokyo | | | JP |
| Sasaki; Katsutoshi | Tokyo | | | JP |
| Endo; Tetsuo | Tokyo | | | JP |
| Tabata; Kazuhiko | Tokyo | | | JP |
| Ozaki; Akio | Tokyo | | | JP |

US-CL-CURRENT: 435/89; 435/183, 435/190, 435/193, 435/194, 435/252.3, 435/252.32, 435/253.6, 435/320.1, 435/325, 435/4, 435/41, 435/6, 435/69.1, 530/350, 536/23.2, 536/23.4, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw De

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| Terms | Documents |
|---|---|
| L7 and L5 | 16 |

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DATE: Monday, December 20, 2004

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|---|---|---|---|
| | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ* | | |
| ☐ | L11 | L10 and GalK | 9 |
| ☐ | L10 | Lgtc | 47 |
| ☐ | L9 | Lgtc and galacto kinase | 0 |
| ☐ | L8 | L7 and l5 | 16 |
| ☐ | L7 | galactokinase and glycosyltransferase | 116 |
| ☐ | L6 | galacto kinase and glycosyltransferase | 0 |
| ☐ | L5 | Galk and glycosyltransferase | 30 |
| | *DB=USPT; PLUR=YES; OP=ADJ* | | |
| ☐ | L4 | L3 | 0 |
| ☐ | L3 | galacto kinase with glycosyltransferase | 0 |
| ☐ | L2 | Galk with glycosyltransferase | 0 |
| ☐ | L1 | Galk with Lgtc | 0 |

END OF SEARCH HISTORY

=> s sugar-nucleotide regenerating enzyme and glycosyltransferase
L1 1 SUGAR-NUCLEOTIDE REGENERATING ENZYME AND GLYCOSYLTRANSFERASE

=> d l1 ibib ab

L1 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07812 BIOTECHDS
TITLE: New vector comprising 2 or more genes encoding sugar-nucleotide regenerating enzymes and one or more gene encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale; involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis
AUTHOR: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO: US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-165735 [16]
AB DERWENT ABSTRACT:

NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding **glycosyltransferase**(s), is new. The genes are operably linked to a promoter.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more **sugar-nucleotide regenerating enzyme** and one or more **glycosyltransferase**; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding **glycosyltransferase**(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.

WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or **glycosyltransferase** enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides.

BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, SusA, GalE, GluT, Ugd or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is LgtB or LgtC. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha-3-N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a **sugar-nucleotide regenerating enzyme** or a **glycosyltransferase** is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 15:08:32 ON 20 DEC 2004

=> file medline hcaplus biosis biotechds scisaerch embase
'SCISAERCH' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files
that are available.  If you have requested multiple files, you can
specify a corrected file name or you can enter "IGNORE" to continue
accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):scisearch
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.42       0.42

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FILE 'HCAPLUS' ENTERED AT 15:09:43 ON 20 DEC 2004
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=> s sugar-nucleotide regenerating enzyme and glycosyltransferase
L1            1 SUGAR-NUCLEOTIDE REGENERATING ENZYME AND GLYCOSYLTRANSFERASE

=> d l1 ibib ab

L1    ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07812  BIOTECHDS
TITLE:            New vector comprising 2 or more genes encoding
                  sugar-nucleotide regenerating enzymes and one or more gene
                  encoding glycosyltransferases, useful for producing
                  glycoconjugates, including oligosaccharides in large-scale;
                     involving vector-mediated gene transfer and expression in
                     host cell for use in oligonucleotide synthesis
AUTHOR:           WANG P G; CHEN X; LIU Z; ZHANG W
PATENT ASSIGNEE:  WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO:      US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO:    US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2003-165735 [16]
AB     DERWENT ABSTRACT:
       NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide
       regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK,
```

PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding **glycosyltransferase**(s), is new. The genes are operably linked to a promoter.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more **sugar -nucleotide regenerating enzyme** and one or more **glycosyltransferase**; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding **glycosyltransferase**(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.
WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or **glycosyltransferase** enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides. BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, SusA, GalE, GluT, Ugd or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is LgtB or LgtC. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha- 3-N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a **sugar- nucleotide regenerating enzyme** or a **glycosyltransferase** is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a **glycosyltransferase**, where the epimerase is UDP-Gal-4-epimerase and the **glycosyltransferase** is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid.
Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell is a yeast cell. One of the heterologous genes in integrated into the genome of the cell. The heterologous genes are encoded within one plasmid.
USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

=> s (Ga1k or GalT or GaIU or Pykf or Ndk or PpK or AcK or PoxB or Ppa or PgM or NagE or Agm1 or glmu or GalNAc kinase or pyrophosphorylase or Ugd or NanA or Cmk or NeuA or A1g2 or Alg1 or SusA or ManB or ManC or phosphomannomutase or Ga1E or GMP or GMD or GFS)
L2 140529 (GA1K OR GALT OR GAIU OR PYKF OR NDK OR PPK OR ACK OR POXB OR PPA OR PGM OR NAGE OR AGML OR GLMU OR GALNAC KINASE OR PYROPHOSPHORYLASE OR UGD OR NANA OR CMK OR NEUA OR A1G2 OR ALGL OR SUSA OR MANB OR MANC OR PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS)

```
=> dup rem l2
140529 ANSWERS REQUESTED EXCEEDS MAXIMUM ALLOWED OF 50000
You may process up to 50,000 answers per command.  Please try to
narrow your search until your resulting L# answer set is within the
maximum number of answers.

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                     22.09      22.51

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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
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                   STN Express with Discover!
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 NEWS  5  NOV 30   PHAR reloaded with additional data
 NEWS  6  DEC 01   LISA now available on STN
 NEWS  7  DEC 09   12 databases to be removed from STN on December 31, 2004
 NEWS  8  DEC 15   MEDLINE update schedule for December 2004
 NEWS  9  DEC 17   ELCOM reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  10 DEC 17   COMPUAB reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  11 DEC 17   SOLIDSTATE reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  12 DEC 17   CERAB reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  13 DEC 17   THREE NEW FIELDS ADDED TO IFIPAT/IFIUDB/IFICDB

 NEWS EXPRESS  OCTOBER 29 CURRENT WINDOWS VERSION IS V7.01A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
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FILE 'HOME' ENTERED AT 15:17:52 ON 20 DEC 2004

=> file medline hcaplus embase biosis biotechds scisearch

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 0.21 | 0.21 |

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FILE 'EMBASE' ENTERED AT 15:18:27 ON 20 DEC 2004


FILE 'BIOSIS' ENTERED AT 15:18:27 ON 20 DEC 2004


FILE 'BIOTECHDS' ENTERED AT 15:18:27 ON 20 DEC 2004


FILE 'SCISEARCH' ENTERED AT 15:18:27 ON 20 DEC 2004


=> s (Ga1k or GalT or GaIU or Pykf or Ndk or PpK or AcK or PoxB or Ppa or PgM) and (LgtB or LgtC)
L1 3 (GA1K OR GALT OR GAIU OR PYKF OR NDK OR PPK OR ACK OR POXB OR PPA OR PGM) AND (LGTB OR LGTC)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2 3 DUP REM L1 (0 DUPLICATES REMOVED)

=> d l2 1-3 ibib ab

L2 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:296912 HCAPLUS Full-text
DOCUMENT NUMBER: 139:291149
TITLE: P1 trisaccharide (Galα1,4Galβ1,4GlcNAc) synthesis by enzyme glycosylation reactions using recombinant Escherichia coli
AUTHOR(S): Liu, Ziye; Lu, Yuquan; Zhang, Jianbo; Pardee, Keith; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA
SOURCE: Applied and Environmental Microbiology (2003), 69(4), 2110-2115
CODEN: AEMIDF; ISSN: 0099-2240

PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 139:291149

AB The frequency of Escherichia coli infection has lead to concerns over pathogenic bacteria in our food supply and a demand for therapeutics. Glycolipids on gut cells serve as receptors for the Shiga-like toxin produced by E. coli. Oligosaccharide moiety analogs of these glycolipids can compete with receptors for the toxin, thus acting as antibacterials. An enzymic synthesis of the P1 trisaccharide (Galα1,4Galβ1,4Glc NAc), one of the oligosaccharide analogs, was assessed in this study. In the proposed synthetic pathway, UDP-glucose was generated from sucrose with an Anabaena sp. sucrose synthase and then converted with an E. coli UDP-glucose 4-epimerase to UDP-galactose. Two mols. of galactose were linked to N-acetylglucosamine subsequently with a Helicobacter pylori β-1,4-galactosyltransferase and a Neisseria meningitidis α-1,4-galactosyltransferase to produce one mol. of P1 trisaccharide. The four enzymes were coexpressed in a single genetically engineered E. coli strain that was then permeabilized and used to catalyze the enzymic reaction. P1 trisaccharide was accumulated up to 50 mM (5.4 g in a 200-mL reaction volume), with a 67% yield based on the consumption of N-acetylglucosamine. This study provides an efficient approach for the preparative-scale synthesis of P1 trisaccharide with recombinant bacteria.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:166313 HCAPLUS Full-text
DOCUMENT NUMBER: 139:163618
TITLE: Production of recombinant xenotransplantation antigen in Escherichia coli
AUTHOR(S): Bettler, Emmanuel; Imberty, Anne; Priem, Bernard; Chazalet, Valerie; Heyraud, Alain; Joziasse, David H.; Geremia, Roberto A.
CORPORATE SOURCE: Centre de Recherches sur les Macromolecules Vegetales (CNRS), Joseph Fourier University, Grenoble, 38041, Fr.
SOURCE: Biochemical and Biophysical Research Communications (2003), 302(3), 620-624
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The synthesis of sufficient amts. of oligosaccharides is the bottleneck for the study of their biol. function and their possible use as drug. As an alternative for chemical synthesis, we propose to use Escherichia coli as a "living factory". We have addressed the production of the Galpα(1-3)Galpβ(1-4)GlcNAc epitope, the major porcine antigen responsible for xenograft rejection. An E. coli strain was generated which simultaneously expresses NodC (to provide the chitin-pentaose acceptor), β(1-4) galactosyltransferase **LgtB**, and bovine α(1-3) galactosyltransferase GstA. This strain produced 0.68 g/L of the heptasaccharide Galpα(1-3)Galpβ(1-4)(GlcNAc)5, which harbors the xenoantigen at its non-reducing end, establishing the feasibility of this approach.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07812 BIOTECHDS
TITLE: New vector comprising 2 or more genes encoding

sugar-nucleotide regenerating enzymes and one or more gene encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale; involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis

AUTHOR: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO: US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-165735 [16]

AB DERWENT ABSTRACT:

NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, **PykF**, **Ndk**, **PpK**, **AcK**, **PoxB**

, **Ppa**, **Pgm**, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), is new. The genes are operably linked to a promoter.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, **PykF**, **Ndk**, **PpK**, **AcK**, **PoxB**

, **Ppa**, **Pgm**, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid. WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or glycosyltransferase enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides. BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, **GalT** and GalU, and a gene encoding **Ndk** , **Ppk**, **PykF**, **PoxB**, **Ndk**, **Ppa**, SusA, GalE, GluT, Ugd or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is **LgtB** or **LgtC.** The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha-3-N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a glycosyltransferase, where the epimerase is UDP-Gal-4-epimerase and the glycosyltransferase is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid. Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell

is a yeast cell. One of the heterologous genes in integrated into the genome of the cell. The heterologous genes are encoded within one plasmid. USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

=> s (NagE or Agml or glmu or GalNAc kinase or pyrophosphorylase or Ugd or NanA ) and (LgtB or LgtC)
L3 9 (NAGE OR AGML OR GLMU OR GALNAC KINASE OR PYROPHOSPHORYLASE OR UGD OR NANA ) AND (LGTB OR LGTC)

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 5 DUP REM L3 (4 DUPLICATES REMOVED)

=> d l4 1-5 ibib ab

L4 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:415845 HCAPLUS Full-text
DOCUMENT NUMBER: 140:89740
TITLE: Efficient synthesis of globoside and isogloboside tetrasaccharides by using β(1→3) N-acetylgalactosaminyltransferase/UDP-N-acetylglucosamine C4 epimerase fusion protein
AUTHOR(S): Shao, Jun; Zhang, Jianbo; Kowal, Przemyslaw; Lu, Yuquan; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA
SOURCE: Chemical Communications (Cambridge, United Kingdom) (2003), (12), 1422-1423
CODEN: CHCOFS; ISSN: 1359-7345
PUBLISHER: Royal Society of Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 140:89740
AB The β(1→3) N-acetylgalactosaminyltransferase/UDP-N- acetylglucosamine C4 epimerase fusion protein was constructed and used in coupled enzymic reactions to synthesize a variety of globotetraose and isoglobotetraose derivs. from the corresponding lactoside acceptors.
REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:191380 HCAPLUS Full-text
DOCUMENT NUMBER: 141:119836
TITLE: The lipo-oligosaccharides of Haemophilus influenzae: An interesting array of characters. [Erratum to document cited in CA139:288666]
AUTHOR(S): Swords, W. Edward; Jones, Paul A.; Apicella, Michael A.
CORPORATE SOURCE: Department of Microbiology and Immunology, Wake Forest University School of Medicine, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2003), 9(5), 336
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. On page 134, column 1, paragraph 1, "A chromosomal locus involved in the biosynthesis of the 2-keto-3-deoctulosonic acid" would be more accurately stated as "A chromosomal locus involved in the exposure of a 2-keto-3-deoctulosonic acid epitope", and "2-keto-deoctulosonic acid" should be "3-deoxy-D-manno-octulosonic acid". In Table 1, the functions for HI0258 (**lgtC**), HI0765 (lpsA) and HI0261 (opsX) have been defined and should not be considered putative (Hood et al., Glycobiol. 2001; 11:957-967; Hood et al., Mol Microbiol 1996; 22:951-966). On page 135, column 1, paragraph 2, RfaE incorrectly referred to as a heptosyltransferase; it is a heptose synthase. On page 137, column 1, paragraph 4, "the acceptor for siaB" is incorrect as SiaB is not a sialyltransferase; this should read "the acceptor for SiaA". On page 138, paragraph 1, the functions for the genes of the lic1 operon would be more accurately described as: licA (choline kinase), licB (putative choline uptake), licC (**pyrophosphorylase**), and licD (phosphorylcholine transferase).

L4 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:293978 HCAPLUS Full-text
DOCUMENT NUMBER: 136:337341
TITLE: Materials and methods to modulate ligand binding/enzymic activity of α/β proteins containing an allosteric regulatory site
INVENTOR(S): Stauton, Donald E.
PATENT ASSIGNEE(S): Icos Corporation, USA
SOURCE: PCT Int. Appl., 163 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002031511 | A2 | 20020418 | WO 2001-US32047 | 20011012 |
| WO 2002031511 | A3 | 20030313 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2425581 | AA | 20020418 | CA 2001-2425581 | 20011012 |
| AU 2002013196 | A5 | 20020422 | AU 2002-13196 | 20011012 |
| US 2003088061 | A1 | 20030508 | US 2001-976935 | 20011012 |
| EP 1325341 | A2 | 20030709 | EP 2001-981560 | 20011012 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| JP 2004511496 | T2 | 20040415 | JP 2002-534845 | 20011012 |
| PRIORITY APPLN. INFO.: | | | US 2000-239750P P | 20001012 |
| | | | WO 2001-US32047 W | 20011012 |

AB Methods of modulating binding between an α/β protein and a binding partner are provided, along with methods of identifying modulators and their use. The methods comprise contacting the α/β protein with an allosteric effector mol. which binds to an allosteric site of the α/β protein and alters the conformation of the α/β protein such that the binding of the α/β protein to a binding partner is modulated. Thus, a primary screen for inhibitors of the

classical pathway complement protein C2 and alternative pathway complement protein factor B involving modifications of standard hemolytic CH50 and AH50 assays in a microtiter plate format was carried out. Lead compds. identified in this screen were submitted to a second screening using purified complement proteins to determine which stage of complement activation the compds. inhibited. Five diaryl sulfides were identified. Numerous other assays, e.g., to identify inhibitors of integrin αEβγ interaction with E cadherin, inhibitors of Rac1 GDP-GTP exchange, or antagonists of E. coli 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, were conducted as well.

L4 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07812 BIOTECHDS
TITLE: New vector comprising 2 or more genes encoding sugar-nucleotide regenerating enzymes and one or more gene encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale; involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis
AUTHOR: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO: US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-165735 [16]
AB DERWENT ABSTRACT:

NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, **NagE**, Agm1, glum, a **GalNAc kinase**, a **pyrophosphorylase**, **Ugd**, **NanA**, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), is new. The genes are operably linked to a promoter.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, **NagE**, Agm1, glum, a **GalNAc kinase**, a **pyrophosphorylase**, **Ugd**, **NanA**, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.

WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or glycosyltransferase enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides. BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, SusA, GalE, GluT, **Ugd** or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is **LgtB** or **LgtC**. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha-3- N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The

fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a glycosyltransferase, where the epimerase is UDP-Gal-4-epimerase and the glycosyltransferase is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid. Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell is a yeast cell. One of the heterologous genes in integrated into the genome of the cell. The heterologous genes are encoded within one plasmid. USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

L4 ANSWER 5 OF 5 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2002301122 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 12042246
TITLE: A new fermentation process allows large-scale production of human milk oligosaccharides by metabolically engineered bacteria.
AUTHOR: Priem Bernard; Gilbert Michel; Wakarchuk Warren W; Heyraud Alain; Samain Eric
CORPORATE SOURCE: Centre de Recherches sur les Macromolecules Vegetales, CNRS, Joseph Fourier University, BP 53, 38041 Grenoble cedex 9 France.
SOURCE: Glycobiology, (2002 Apr) 12 (4) 235-40.
Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20020604
Last Updated on STN: 20021214
Entered Medline: 20021127

AB When fed to a beta-galactosidase-negative (lacZ(-)) Escherichia coli strain that was grown on an alternative carbon source (such as glycerol), lactose accumulated intracellularly on induction of the lactose permease. We showed that intracellular lactose was efficiently glycosylated when genes of glycosyltransferase that use lactose as acceptor were expressed. High-cell-density cultivation of lacZ(-) strains that overexpressed the beta 1,3 N acetyl glucosaminyltransferase lgtA gene of Neisseria meningitidis resulted in the synthesis of 6 g x L(-1) of the expected trisaccharide (GlcNAc beta 1-3Gal beta 1-4Glc). When the beta 1,4 galactosyltransferase **lgtB** gene of N. meningitidis was coexpressed with lgtA, the trisaccharide was further converted to lacto-N-neotetraose (Gal beta 1-4GlcNAc beta 1-3Gal beta 1-4Glc) and lacto-N-neoheaxose with a yield higher than 5 g x L(-1). In a similar way, the **nanA**(-) E. coli strain that was devoid of NeuAc aldolase activity accumulated NeuAc on induction of the NanT permease and the lacZ(-) **nanA**(-) strain that overexpressed the N. meningitidis genes of the alpha2,3 sialyltransferase and of the CMP-NeuAc synthase efficiently produced sialyllactose (NeuAc alpha 2-3Gal beta 1-4Glc) from exogenous NeuAc and lactose.

```
=> s (Cmk or NeuA or A1g2 or Algl or SusA or ManB or ManC) and (LgtB or LgtC)
L5            3 (CMK OR NEUA OR A1G2 OR ALGL OR SUSA OR MANB OR MANC) AND (LGTB
                OR LGTC)

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            3 DUP REM L5 (0 DUPLICATES REMOVED)

=> d l6 1-3 ibib ab

L6   ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:296912  HCAPLUS Full-text
DOCUMENT NUMBER:          139:291149
TITLE:                    P1 trisaccharide (Galα1,4Galβ1,4GlcNAc)
                          synthesis by enzyme glycosylation reactions using
                          recombinant Escherichia coli
AUTHOR(S):                Liu, Ziye; Lu, Yuquan; Zhang, Jianbo; Pardee, Keith;
                          Wang, Peng George
CORPORATE SOURCE:         Department of Chemistry, Wayne State University,
                          Detroit, MI, 48202, USA
SOURCE:                   Applied and Environmental Microbiology (2003), 69(4),
                          2110-2115
                          CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER:                American Society for Microbiology
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
OTHER SOURCE(S):          CASREACT 139:291149
AB    The frequency of Escherichia coli infection has lead to concerns over
      pathogenic bacteria in our food supply and a demand for therapeutics.
      Glycolipids on gut cells serve as receptors for the Shiga-like toxin produced
      by E. coli. Oligosaccharide moiety analogs of these glycolipids can compete
      with receptors for the toxin, thus acting as antibacterials. An enzymic
      synthesis of the P1 trisaccharide (Galα1,4Galβ1,4Glc NAc), one of the
      oligosaccharide analogs, was assessed in this study. In the proposed
      synthetic pathway, UDP-glucose was generated from sucrose with an Anabaena sp.
      sucrose synthase and then converted with an E. coli UDP-glucose 4-epimerase to
      UDP-galactose. Two mols. of galactose were linked to N-acetylglucosamine
      subsequently with a Helicobacter pylori β-1,4-galactosyltransferase and a
      Neisseria meningitidis α-1,4-galactosyltransferase to produce one mol. of P1
      trisaccharide. The four enzymes were coexpressed in a single genetically
      engineered E. coli strain that was then permeabilized and used to catalyze the
      enzymic reaction. P1 trisaccharide was accumulated up to 50 mM (5.4 g in a
      200-mL reaction volume), with a 67% yield based on the consumption of N-
      acetylglucosamine. This study provides an efficient approach for the
      preparative-scale synthesis of P1 trisaccharide with recombinant bacteria.
REFERENCE COUNT:          51    THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6    ANSWER 2 OF 3  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07812  BIOTECHDS
TITLE:            New vector comprising 2 or more genes encoding
                  sugar-nucleotide regenerating enzymes and one or more gene
                  encoding glycosyltransferases, useful for producing
                  glycoconjugates, including oligosaccharides in large-scale;
                     involving vector-mediated gene transfer and expression in
                     host cell for use in oligonucleotide synthesis
AUTHOR:           WANG P G; CHEN X; LIU Z; ZHANG W
```

PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO: US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-165735 [16]

AB DERWENT ABSTRACT:

NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, **Cmk**, **NeuA**, Alg2, Alg1, **SusA**, **ManB**, **ManC**, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), is new. The genes are operably linked to a promoter.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, **Cmk**, **NeuA**, Alg2, Alg1, **SusA**, **ManB**, **ManC**, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.

WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or glycosyltransferase enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides. BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, **SusA**, GalE, GluT, Ugd or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is **LgtB** or **LgtC**. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha-3- N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a glycosyltransferase, where the epimerase is UDP-Gal-4-epimerase and the glycosyltransferase is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid. Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell is a yeast cell. One of the heterologous genes in integrated into the genome of the cell. The heterologous genes are encoded within one plasmid.

USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

L6 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:594853 HCAPLUS Full-text
DOCUMENT NUMBER: 135:317504
TITLE: Transferring a Biosynthetic Cycle into a Productive Escherichia coli Strain: Large-Scale Synthesis of Galactosides
AUTHOR(S): Chen, Xi; Zhang, Jianbo; Kowal, Przemek; Liu, Ziye; Andreana, Peter R.; Lu, Yuquan; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA
SOURCE: Journal of the American Chemical Society (2001), 123(36), 8866-8867
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 135:317504

AB A simple and efficient system for the large scale production of galactosides is described which involves introducing a synthetic 3 gene cluster into Escherichia coli already possessing a recombinant α-1,3-galactosyltransferase. The genes encoding sucrose synthase, UDP-galactose-4-epimerase, and α-1,4-galactosyltransferase were incorporated into a temperature sensitive pLDR20 plasmid and transformed into E. coli. Recombinant E. coli cells were produced by a fed-batch fermentation, harvested, and permeabilized by heat treatment. Whole cells of heat treated E. coli were then used to produce two galactosides, globotriose and α-galactopyranosyl-1→3-lactose from lactose, sucrose and UDP. The biosynthetic system permits the recycling of UDP while producing fructose as a byproduct.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s (phosphomannomutase or Ga1E or GMP or GMD or GFS) and (LgtB or LgtC)
L7 1 (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (LGTB OR LGTC)

=> d l7

L7 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
AN 2003-07812 BIOTECHDS Full-text
TI New vector comprising 2 or more genes encoding sugar-nucleotide regenerating enzymes and one or more gene encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale;
involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis
AU WANG P G; CHEN X; LIU Z; ZHANG W
PA WANG P G; CHEN X; LIU Z; ZHANG W
PI US 2002132320 19 Sep 2002
AI US 2001-758525 10 Jan 2001
PRAI US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DT Patent
LA English
OS WPI: 2003-165735 [16]

=> s (phosphomannomutase or Ga1E or GMP or GMD or GFS) and (Lgtf, Alg5 or DUGT )
L8 1 (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (LGTF, ALG5 OR DUGT )

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=> s (phosphomannomutase or Ga1E or GMP or GMD or GFS) and (N-acetylglucosaminyl
transferase); UDP-GalNAc:2'-fucosylgalactiside-I-3-N-acetylgalactosaminyl
transferase; UGT2B7
L9          11 (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (N-ACETYLG
               LUCOSAMINYL TRANSFERASE)


UDP-GALNAC:2'-FUCOSYLGALACTISIDE-I-3-N-ACETYLGALACTOSAMINYL IS NOT A RECOGNIZED
COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s (phosphomannomutase or Ga1E or GMP or GMD or GFS) and (N-acetylglucosaminyl
transferase or UDP-GalNAc-fucosylgalactiside-1-3-N-acetylgalactosaminyl transferase
or UGT2B7)
L10         11 (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (N-ACETYLG
               LUCOSAMINYL TRANSFERASE OR UDP-GALNAC-FUCOSYLGALACTISIDE-1-3-N-A
               CETYLGALACTOSAMINYL TRANSFERASE OR UGT2B7)

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11           7 DUP REM L10 (4 DUPLICATES REMOVED)

=> d l11 1-7 ibib ab

L11  ANSWER 1 OF 7  SCISEARCH  COPYRIGHT (c) 2004 The Thomson Corporation.  on
     STN
ACCESSION NUMBER:    2004:489010  SCISEARCH Full-text
THE GENUINE ARTICLE: 822JJ
TITLE:               Glycosylation of the receptor guanylate cyclase C: role in
                     ligand binding and catalytic activity
AUTHOR:              Ghanekar Y; Chandrashaker A; Tatu U; Visweswariah S S
                     (Reprint)
CORPORATE SOURCE:    Indian Inst Sci, Dept Mol Reprod Dev & Genet, Bangalore
                     560012, Karnataka, India (Reprint); Indian Inst Sci, Dept
                     Biochem, Bangalore 560012, Karnataka, India
COUNTRY OF AUTHOR:   India
SOURCE:              BIOCHEMICAL JOURNAL, (1 MAY 2004) Vol. 379, Part 3, pp.
                     653-663.
                     Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N
                     3AJ, ENGLAND.
                     ISSN: 0264-6021.
DOCUMENT TYPE:       Article; Journal
LANGUAGE:            English
REFERENCE COUNT:     44
                    *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB       GC-C (guanylate cyclase C) is the receptor for heat-stable enterotoxins,
         guanylin and uroguanylin peptides. Ligand binding to the extracellular
         domain of GC-C activates the guanylate cyclase domain leading to
         accumulation of cGMP. GC-C is expressed as differentially glycosylated
         forms in HEK-293 cells (human embryonic kidney-293 cells). In the present
         study, we show that the 145 kDa form of GC-C contains sialic acid and
         galactose residues and is present on the PM (plasma membrane) of cells,
         whereas the 130 kDa form is a high mannose form that is resident in the
         endoplasmic reticulum and serves as the precursor for the PM-associated
         form. Ligand-binding affinities of the differentially glycosylated forms
         are similar, indicating that glycosylation of GC-C does not play a role in
         direct ligand interaction. However, ligand-stimulated guanylate cyclase
         activity was observed only for the fully mature form of the receptor
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present on the PM, suggesting that glycosylation had a role to play in imparting a conformation to the receptor that allows ligand stimulation. Treatment of cells at 20 degreesC led to intracellular accumulation of a mature glycosylated form of GC-C that now showed ligand-stimulated guanylate cyclase activity, indicating that localization of GC-C was not critical for its catalytic activity. To determine if complex glycosylation was required for ligand-stimulated activation of GC-C, the receptor was expressed in HEK-293 cells that were deficient in **N-acetylglucosaminyl-transferase** 1. This minimally glycosylated form of the receptor was expressed on the cell surface and could bind a ligand with an affinity comparable with the 145 kDa form of the receptor. However, this form of the receptor was poorly activated by the ligand. Therefore our studies indicate a novel role for glycosidic modification of GC-C during its biosynthesis, in imparting subtle conformational changes in the receptor that allow for ligand-mediated activation and perhaps regulation of basal activity.

L11 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2003:463249 BIOSIS Full-text
DOCUMENT NUMBER: PREV200300463249
TITLE: Congenital disorders of glycosylation (CDG): It's all in it!.
AUTHOR(S): Jaeken, J. [Reprint Author]
CORPORATE SOURCE: Department of Pediatrics, Centre for Metabolic Disease, University Hospital Gasthuisberg, Herestraat 49, B-3000, Leuven, Belgium
jaak.jaeken@uz.kuleuven.ac.be
SOURCE: Journal of Inherited Metabolic Disease, (2003) Vol. 26, No. 2-3, pp. 99-118. print.
ISSN: 0141-8955 (ISSN print).
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Oct 2003
Last Updated on STN: 8 Oct 2003

AB Congenital disorders of glycosylation (CDGs) are due to defects in the synthesis of the glycan moiety of glycoproteins or other glycoconjugates. This review is devoted mainly to the clinical aspects of protein glycosylation defects. There are two main types of protein glycosylation: N-glycosylation and O-glycosylation. N-glycosylation generally consists of an assembly pathway (in cytosol and endoplasmic reticulum) and a processing pathway (in endoplasmic reticulum and Golgi). O-glycosylation lacks a processing pathway but is otherwise more complex. Sixteen disease-causing defects are known in protein glycosylation: 12 in N-glycosylation and four in O-glycosylation. The N-glycosylation defects comprise eight assembly defects (CDG-I) designated CDG-Ia to CDG-Ih, and four processing defects (CDG-II) designated CDG-IIa to CDG-IId. By far the most frequent is CDG-Ia (**phosphomannomutase**-2 deficiency). It affects the nervous system and many other organs. Its clinical expression varies from extremely severe to very mild (and thus probably underdiagnosed). The most interesting disease in this group is CDG-Ib (phosphomannose isomerase deficiency) because it is so far the only efficiently treatable CDG (mannose treatment). It has a hepatic-intestinal presentation. The O-glycosylation defects comprise two O-xylosylglycan defects (a progeroid variant of Ehlers-Danlos syndrome and the multiple exostoses syndrome) and two O-mannosylglycan defects (Walker-Warburg syndrome and muscle-eye-brain disease). All known CDGs have a recessive inheritance except for multiple exostoses syndrome, which is dominantly inherited. There is a rapidly growing group of putative CDGs with a large spectrum of clinical presentations (CDG-x). Serum transferrin iso-electrofocusing remains the cornerstone of the screening for N-glycosylation defects associated with

sialic acid deficiency. Abnormal patterns can be grouped in to type 1 and type 2. However, a normal pattern does not exclude these defects. Screening for the other CDGs is much more difficult, particularly when the defect is organ- or system-restricted. The latter group promises to become an important new chapter in CDG. It is concluded that CDGs will eventually cover the whole clinical spectrum of paediatric and adult disease manifestations.

L11 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:937303 HCAPLUS Full-text
DOCUMENT NUMBER: 138:20443
TITLE: Endocrine disruptor screening using DNA chips of endocrine disruptor-responsive genes
INVENTOR(S): Kondo, Akihiro; Takeda, Takeshi; Mizutani, Shigetoshi; Tsujimoto, Yoshimasa; Takashima, Ryokichi; Enoki, Yuki; Kato, Ikunoshin
PATENT ASSIGNEE(S): Takara Bio Inc., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 386 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | | DATE |
|---|---|---|---|---|---|
| JP 2002355079 | A2 | 20021210 | JP 2002-69354 | | 20020313 |
| PRIORITY APPLN. INFO.: | | | JP 2001-73183 | A | 20010314 |
| | | | JP 2001-74993 | A | 20010315 |
| | | | JP 2001-102519 | A | 20010330 |

AB A method and kit for detecting endocrine-disrupting chems. using DNA microarrays are claimed. The method comprises preparing a nucleic acid sample containing mRNAs or cDNAs originating in cells, tissues, or organisms which have been brought into contact with a sample containing the endocrine disruptor. The nucleic acid sample is hybridized with DNA microarrays having genes affected by the endocrine disruptor or DNA fragments originating in these genes have been fixed. The results obtained are then compared with the results obtained with the control sample to select the gene affected by the endocrine disruptor. Genes whose expression is altered by tri-Bu tin, 4-octaphenol, 4-nonylphenol, di-N-Bu phthalate, dichlorohexyl phthalate, octachlorostyrene, benzophenone, diethylhexyl phthalate, diethylstilbestrol (DES), and 17-β estradiol (E2), were found in mice by DNA chip anal.

L11 ANSWER 4 OF 7 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07812 BIOTECHDS
TITLE: New vector comprising 2 or more genes encoding sugar-nucleotide regenerating enzymes and one or more gene encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale; involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis
AUTHOR: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W
PATENT INFO: US 2002132320 19 Sep 2002
APPLICATION INFO: US 2001-758525 10 Jan 2001
PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-165735 [16]

AB DERWENT ABSTRACT:
NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a **phosphomannomutase**, GalE, **GMP**, **GMD**, and **GFS**; and (b) one or more genes encoding glycosyltransferase(s), is new. The genes are operably linked to a promoter.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from Galk, GalkT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a **phosphomannomutase**, GalE, **GMP**, **GMD**, and **GFS**; and (b) one or more genes encoding glycosyltransferase(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.
WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or glycosyltransferase enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides. BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, SusA, GalE, GluT, Ugd or **UGT2B7**. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an **N-acetylglucosaminyl transferase**, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is LgtB or LgtC. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNac:2'-fucosylgalactoside-alpha-3- N-acetylgalactosaminyl transferase. The glucuronyltransferase is **UGT2B7**. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alf1 or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a glycosyltransferase, where the epimerase is UDP-Gal-4-epimerase and the glycosyltransferase is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid.
Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell is a yeast cell. One of the heterologous genes in integrated into the genome of the cell. The heterologous genes are encoded within one plasmid.
USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

L11 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:668499 HCAPLUS Full-text
DOCUMENT NUMBER: 130:63448
TITLE: Biosynthetic enzymes of tetrahydrolimipterin from green sulfur bacterium Chlorobium limicola
AUTHOR(S): Kang, Dongmin; Kim, Sangjoon; Yim, Jeongbin
CORPORATE SOURCE: Department Microbiology, Seoul National University, Seoul, 151, S. Korea

SOURCE: Pteridines (1998), 9(2), 69-84
CODEN: PTRDEO; ISSN: 0933-4807
PUBLISHER: International Society of Pteridinology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Based on the structure of limipterin, the biosynthetic pathway for the newly identified pterin glycoside was investigated. It was demonstrated that tetrahydrolimipterin (H4-limipterin) can be synthesized from GTP by the enzymes GTP cyclohydrolase I, 6-pyruvoyltetrahydropterin (PTP) synthase, sepiapterin reductase, and limipterin synthase, present in the extract of C. limicola. Limipterin synthase (UDP-N-acetylglucosamine:5,6,7,8- tetrahydro-L-biopterin 2'-O-β-**N-acetylglucosaminyl transferase**) catalyzed the condensation of tetrahydrobiopterin (H4-biopterin) with UDP-N-acetylglucosamine in the presence of dithiothreitol and MnCl2. It could also produce D-tepidopterin, [1-O-(D-threo-biopterin-2'-yl)-β-N-acetylglucosamine] when 5,6,7,8-tetrahydro-D-threobiopterin and UDP-N-acetylglucosamine were used as substrates. Substrate analogs such as UTP, UDP and UDP-N-acetylgalactosamine inhibit the enzyme activity. The K values for tetrahydrobiopterin and UDP-N-acetylglucosamine were 42.2 μM and 124.3 μM, resp. Optimum pH and temperature were pH 8.0 and 37°. The mol. weight of the enzyme was calculated to be 46,300 dalton from a calibrated Superdex 75 and the subunit mol. weight was estimated at 46,000 dalton by SDS-PAGE. These results suggest that limipterin synthase exists as a monomer. Biosynthetic intermediates of H4-limipterin such as H2NTP, 6-PTP, and H4-biopterin were identified in vitro using purified GTP cyclohydrolase I, PTP synthase, sepiapterin reductase, and limipterin synthase. From the HPLC and TLC analyses of the enzymic intermediates, it could be concluded that H4-limipterin comes from GTP by way of H4-biopterin in C. limicola.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 6 OF 7 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998115443 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9455908
TITLE: Hypoglycosylation of a brain glycoprotein (beta-trace protein) in CDG syndromes due to **phosphomannomutase** deficiency and **N-acetylglucosaminyl-transferase** II deficiency.
AUTHOR: Pohl S; Hoffmann A; Rudiger A; Nimtz M; Jaeken J; Conradt H S
CORPORATE SOURCE: Gesellschaft fur Biotechnologische Forschung, Department of Protein Glycosylation, Braunschweig, Germany.
SOURCE: Glycobiology, (1997 Dec) 7 (8) 1077-84.
Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 20000303
Entered Medline: 19980302

AB Human beta-trace protein is a major intrathecally synthesized polypeptide constituent of human cerebrospinal fluid. We have previously shown that this protein is almost quantitatively modified with biantennary complex-type N-linked oligosaccharides which show "brain-type" glycosylation characteristics (Hoffmann,A. et al., J. Neurochem., 63, pp. 2185-2191, 1994). In the present study human beta-trace protein from the cerebrospinal fluid (CSF) of patients with carbohydrate-deficient glycoprotein syndrome (CDGS) due to

**phosphomannomutase** (PMM) deficiency and N-acetyl-glucosaminyltransferase II (GlcNAc-T II) deficiency as well as from control individuals was studied by Western blot analysis. The protein from pooled CSFs was purified by immunoaffinity chromatography. The protein from the five patients with CDGS PMM deficiency showed three protein bands upon SDS-PAGE analysis corresponding to the di-, mono-, and unglycosylated polypeptide forms. Carbohydrate structural analysis of the enzymatically liberated N-glycans was performed applying mapping by HPAEC-PAD, methylation analysis as well as MALD/TOF-MS. Essentially identical oligosaccharide structures were detected in beta-TP from type I patients and control adult pooled CSF. The beta-trace protein from two patients with GlcNAc-T II deficiency showed a single di-N-glycosylated protein band with a significantly lower molecular weight than the di-glycosylated polypeptide from control patients and the beta-trace protein from pooled adult CSF. Beta-TP from GlcNAc-T II deficiency patients shared only three oligosaccharides out of the 13 observed in beta-TP from controls or patients with PMM deficiency. The major oligosaccharide structures of the glycoprotein from patients with GlcNAc-T II deficiency were found to be monoantennary asialo- or monosialylated lactosamine-type chains with proximal fucose and bisecting GlcNAc.

L11 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:469277 HCAPLUS Full-text
DOCUMENT NUMBER: 109:69277
TITLE: Glycoprotein synthesis in maize endosperm cells. The nucleoside diphosphate-sugar:dolichol-phosphate glycosyltransferases
AUTHOR(S): Riedell, Walter E.; Miernyk, Jan A.
CORPORATE SOURCE: Seed Biosynth. Res. Univ., United States Dep. Agric., Peoria, IL, 61604, USA
SOURCE: Plant Physiology (1988), 87(2), 420-6
CODEN: PLPHAY; ISSN: 0032-0889
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Microsomal membrane prepns. from maize (Zea mays L., inbred A636) endosperm cultures contained enzymes that transferred sugar moieties from UDP-N-acetylglucosamine, GDP-mannose, and UDP-glucose to dolichol-phosphate. These enzyme activities were characterized with respect to detergent and pH optima, substrate kinetic consts., and product and antibiotic inhibition consts. The products of the N-acetylglucosamine transferases were N-acetylglucosamine-pyrophosphoryl-dolichol and N,N'-diacetyl-chitobiosyl-pyrophosphoryl-dolichol, and the product of the mannose transferase was mannosyl-phosphoryl-dolichol. A large proportion of the products of the glucose transferase activity was stable to mild acid hydrolysis. However, the proportion that was labile was identified as glucosyl-phosphoryl-dolichol. Rate zonal sedimentation and isopycnic banding in linear sucrose d. gradients indicated that the glycosyltransferase activities were in the endoplasmic reticulum. The glycosyltransferases were not solubilized by 500 mM KCl or by sequential washes with tris(hydroxymethyl)aminomethane and H2O, treatments that release peripheral membrane proteins. Solubilization was achieved with low concns. of Triton X-100. When sealed microsomal vesicles were incubated with trypsin for 30 min in absence of detergent, **N- acetylglucosaminyl-transferase** activity was substantially reduced, while the activity of the glucosyl-transferase was somewhat reduced. Mannose transferase was resistant to inactivation by trypsin unless Triton was present.

=> d his

(FILE 'HOME' ENTERED AT 15:17:52 ON 20 DEC 2004)

FILE 'MEDLINE, HCAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 15:18:27 ON 20 DEC 2004

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L1             3 S (GA1K OR GALT OR GAIU OR PYKF OR NDK OR PPK OR ACK OR POXB OR
L2             3 DUP REM L1 (0 DUPLICATES REMOVED)
L3             9 S (NAGE OR AGML OR GLMU OR GALNAC KINASE OR PYROPHOSPHORYLASE O
L4             5 DUP REM L3 (4 DUPLICATES REMOVED)
L5             3 S (CMK OR NEUA OR A1G2 OR ALGL OR SUSA OR MANB OR MANC) AND (LG
L6             3 DUP REM L5 (0 DUPLICATES REMOVED)
L7             1 S (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (LGTB O
L8             1 S (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (LGTF,
L9            11 S (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (N-ACET
L10           11 S (PHOSPHOMANNOMUTASE OR GA1E OR GMP OR GMD OR GFS) AND (N-ACET
L11            7 DUP REM L10 (4 DUPLICATES REMOVED)

=> log y
```

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 90.02 | 90.23 |

| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| CA SUBSCRIBER PRICE | -7.00 | -7.00 |

STN INTERNATIONAL LOGOFF AT 15:38:30 ON 20 DEC 2004